

"Focus is key to Success, इसलिए अपनी पढ़ाई में मन लगाओ और अपने सपनों को सच करने की दिशा में कदम बढ़ाओ।"

CSIR NET – Life Science

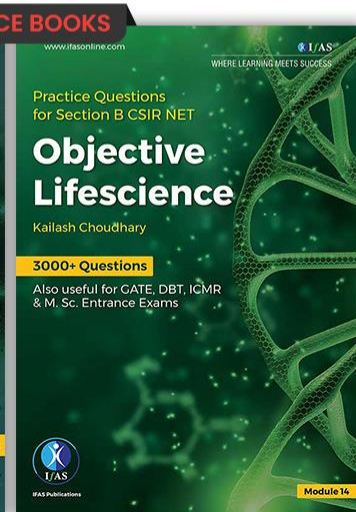
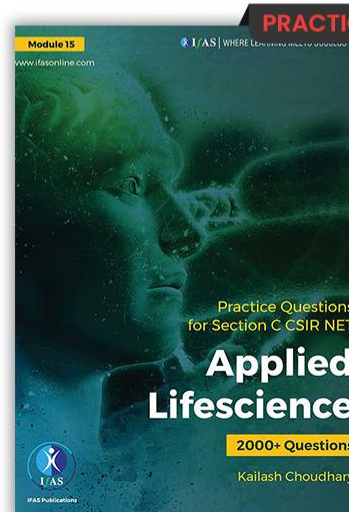
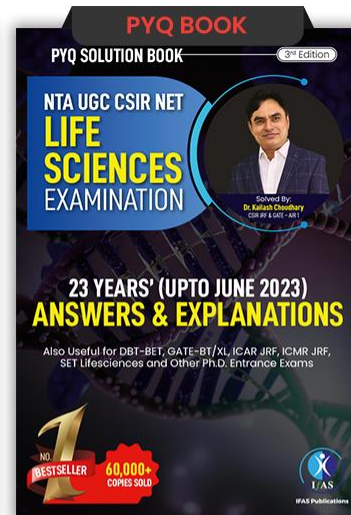
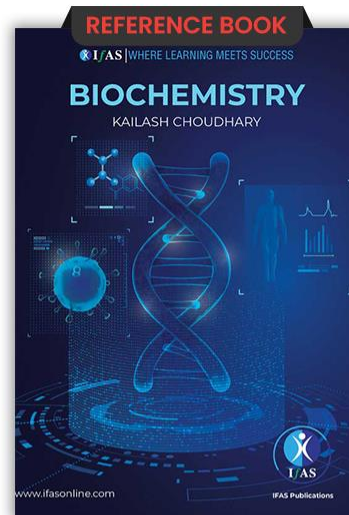
Unit 1: Biochemistry

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Proteins








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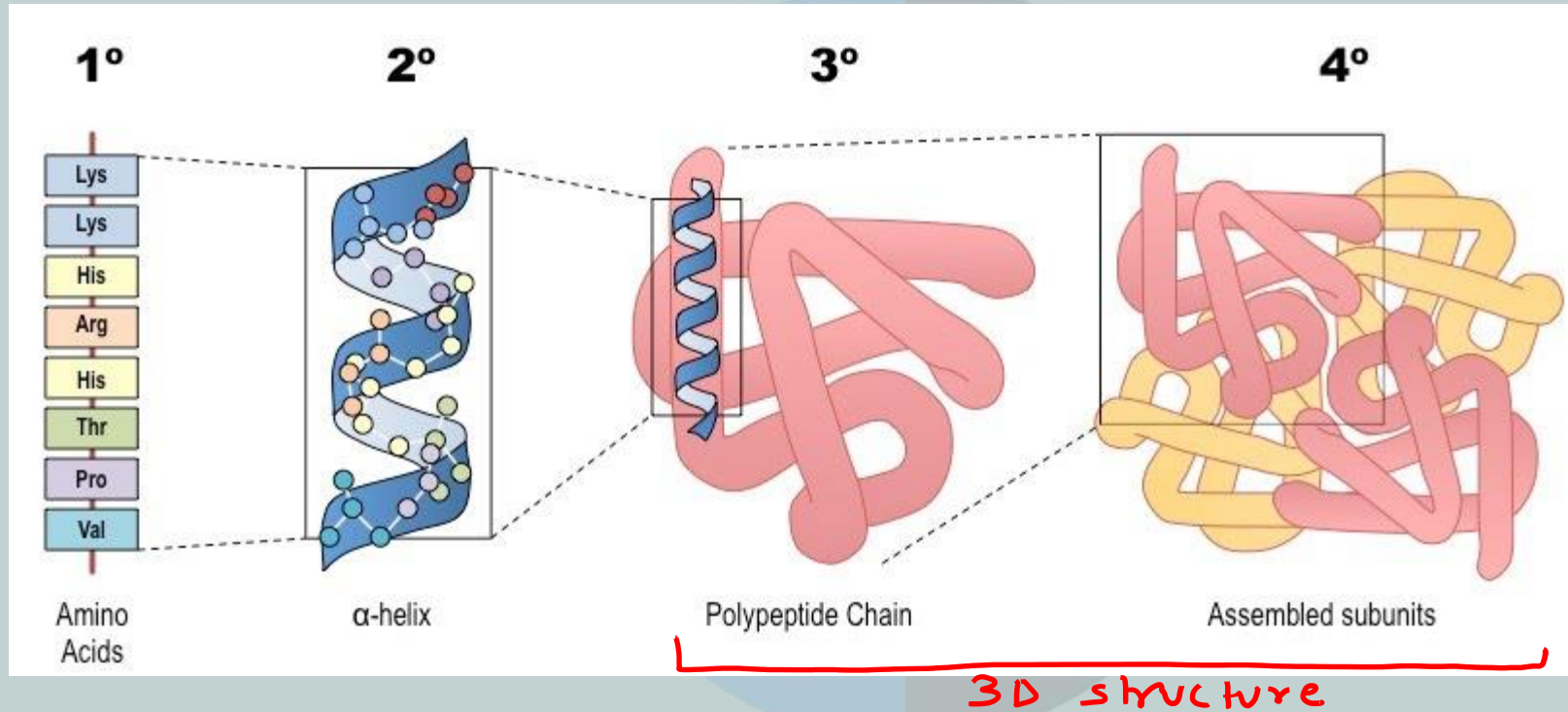
Points to be covered in this Lecture

-  Ramachandran Plot
-  Secondary Structure of Proteins
-  Super secondary structure (motif)
-  Domain and Fold
-  Myoglobin and Haemoglobin
-  Keratin and Collagen
-  Protein folding
-  Stability and solubility of Protein



Imp

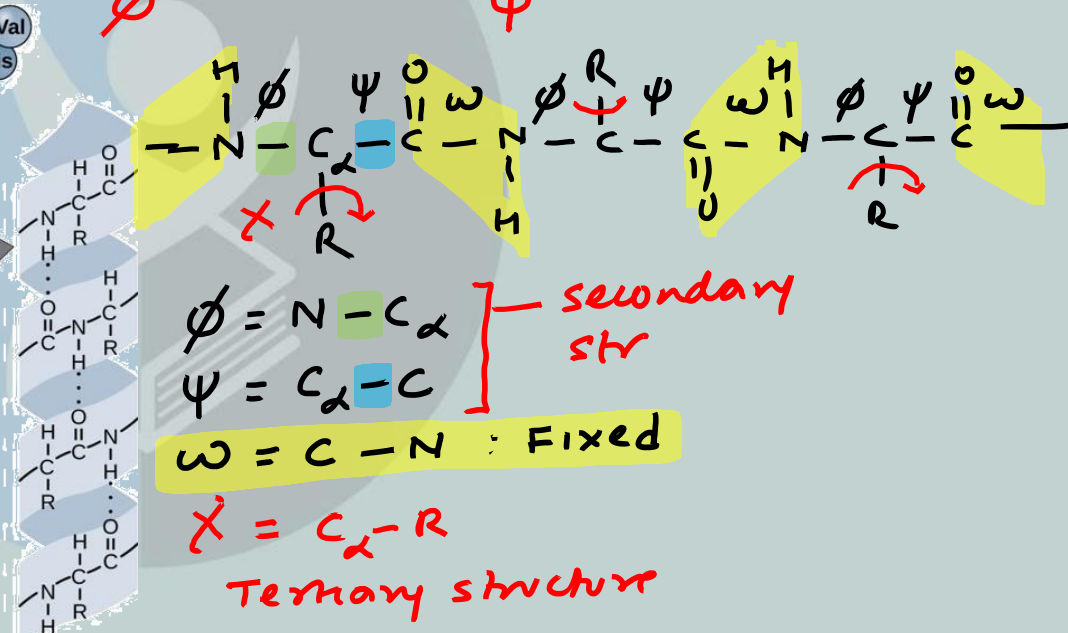
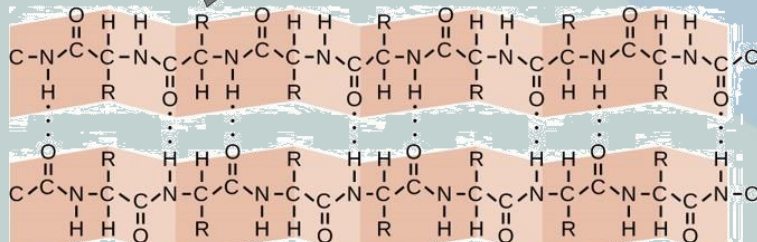
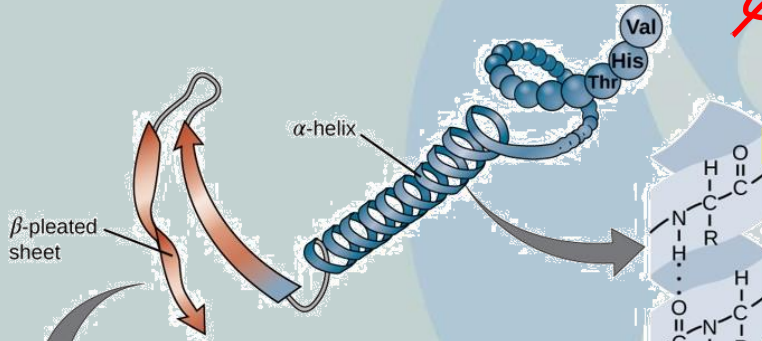
Types of Protein Structure



Secondary Structure of proteins

✓ The local conformation of its backbone

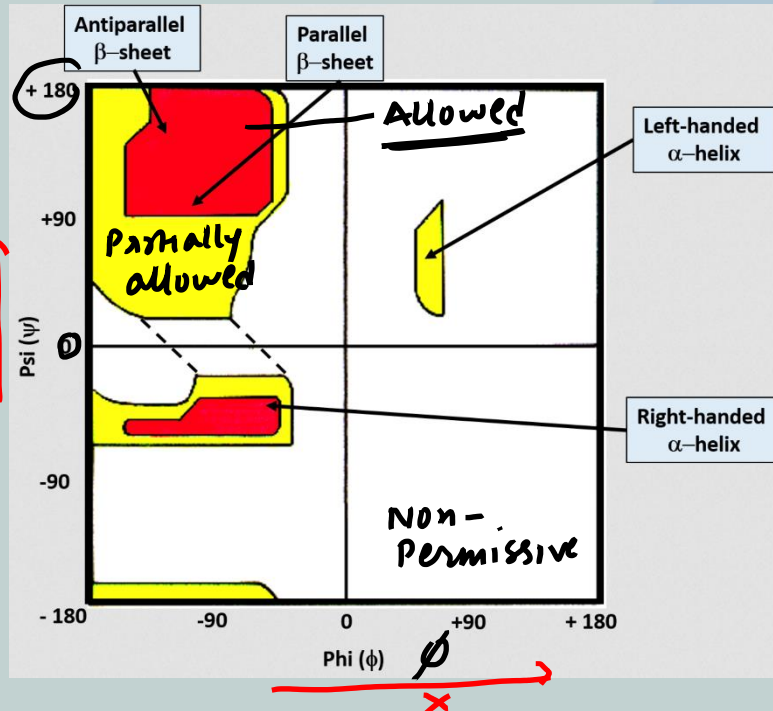
✓ Due to rotation at torsion/dihedral angles): N - C α bond and the C α - C' bond





RAMACHANDRAN PLOT

It shows the possible conformations of ϕ and ψ angles for a polypeptide and prediction about secondary structures

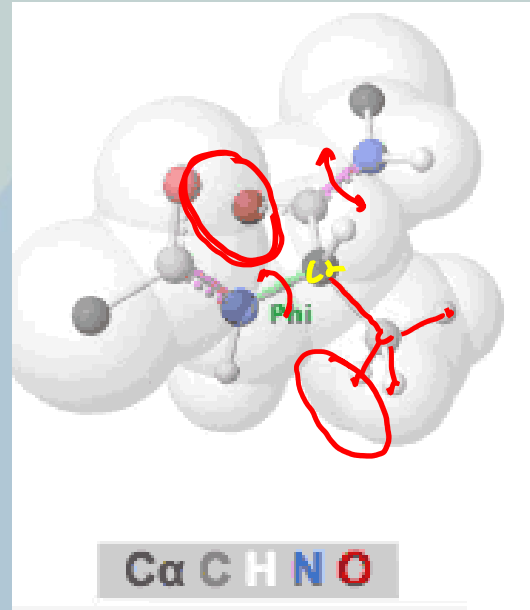
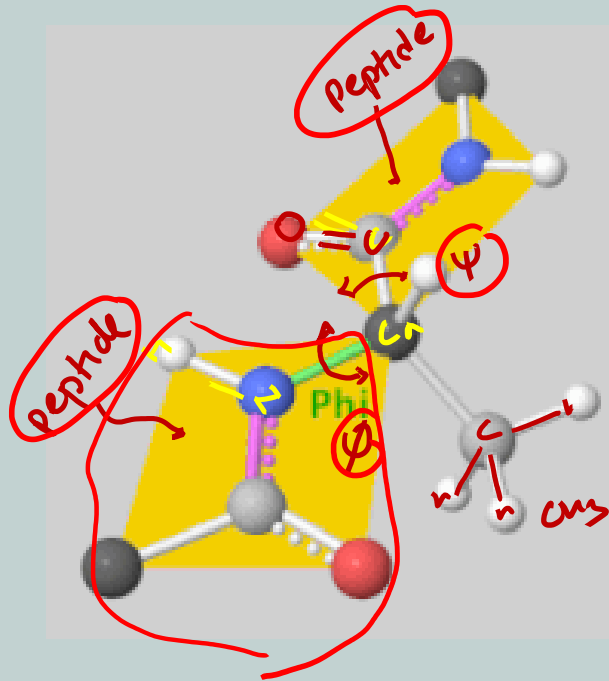


- Different aa can adopt different ϕ & ψ value

Possible : 0 to +180°
 N-C α (ϕ) 0 to -180°
 C α -C (ψ)

- All values are not allowed due to steric hindrance

when 2 atoms are closer than vanderwaal contact distance



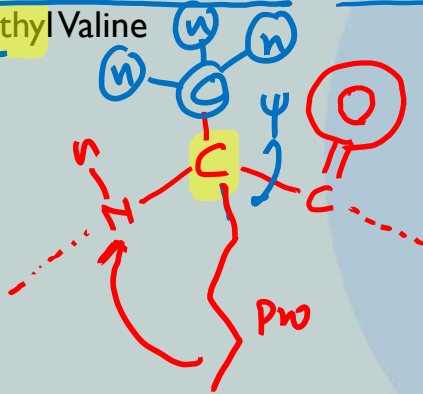


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Three-quarters of the possible (ϕ , ψ) combinations are excluded simply by local steric clashes.

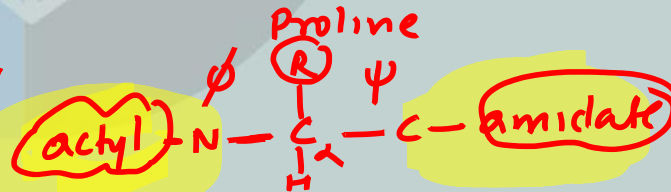
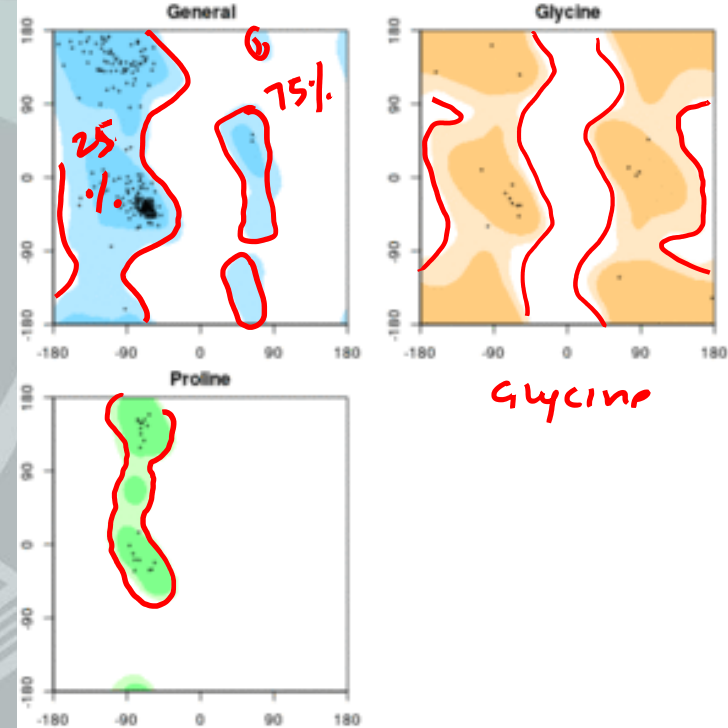
Occupancy on Ramachandran Plot

Glycine > Unbranched Amino Acid > Branched Amino Acid > Proline > α -methyl Valine



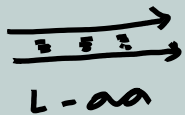
ϕ , ψ Dihedral angles:

X-Ray crystallography

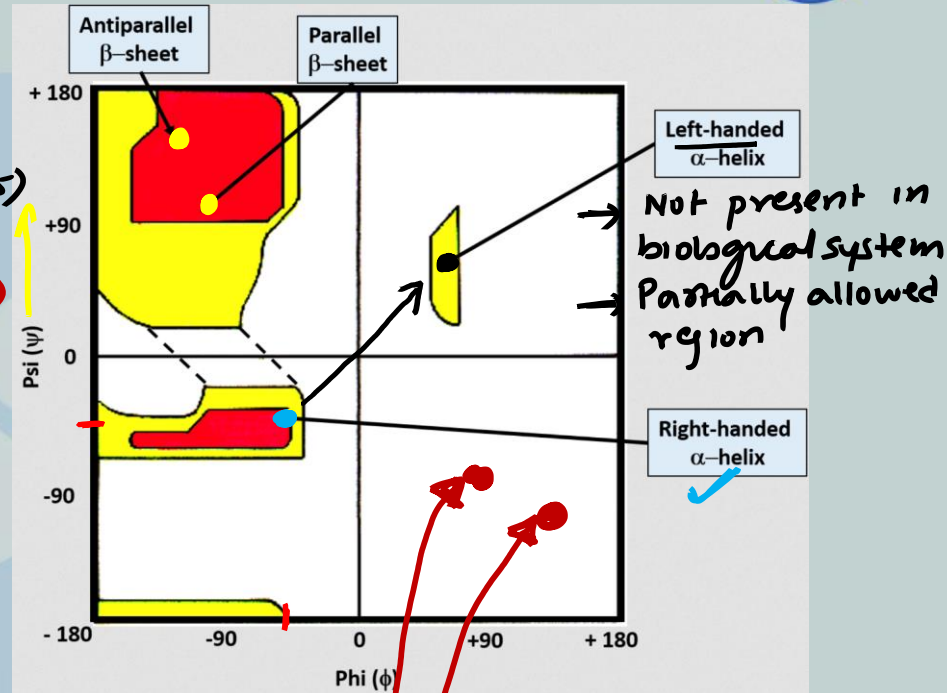


Secondary structure	ϕ	ψ
RH α -helix (L-aa)	-57°	-47°
LH α -helix (D-aa)	$+57$	$+47$
Parallel β -sheets	$-119 (-120)$	$+113 (+115)$
Anti Parallel β -sheets	$-139 (-140)$	$+135$
3_{10} helix	$-49 (-50)$	$-26 (-25)$
π -helix	-55	-70
Collagen Tripple helix	-51	$+153$

	ϕ	ψ	
L-aa	-57	-47°	oooo
D-aa	$+57$	$+47^\circ$	oooo



D-aa
 $\phi = +120$
 $\psi = -115$
 $\phi = +140$ $\psi = -135^\circ$



Parallel β -sheet

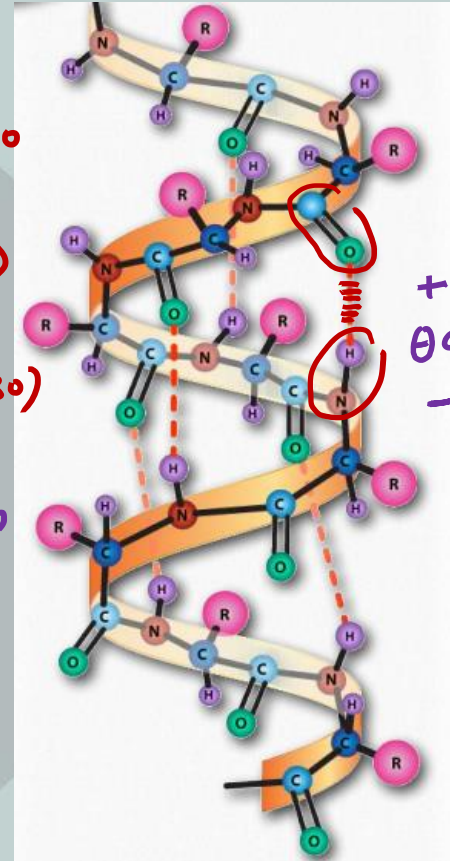
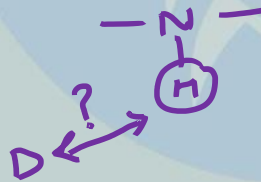
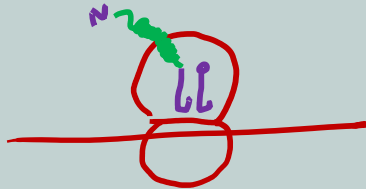
Antiparallel β -sheets



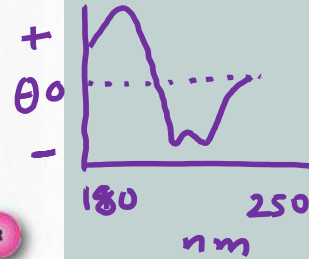
RH Alpha-Helix: Imp ✓

- ✓ Right handed (L-amino acids)
- ✓ One helix has 3.6 amino acids
- ✓ Helix height 5.4 Å (0.54 nm)
- ✓ Distance between two amino acids = 1.5 Å
- ✓ ϕ , ψ dihedral angles near -57°, -47° (-60 to -30)
- ✓ Intra Chain hydrogen bonding
- ✓ First formed secondary structure, *most common*
- Slow exchange amide H/D in NMR

$$\frac{5.4 \text{ Å}}{3.6 \text{ aa}} = 1.5 \text{ Å} \quad (0.15 \text{ nm})$$



Far UV
CD Spectro-
scopy



(+) 192 nm
(-) 208 nm
222 nm

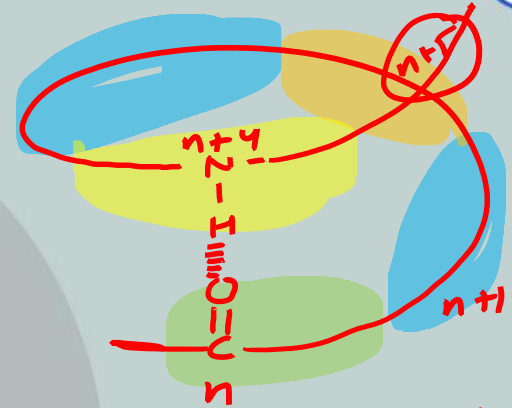
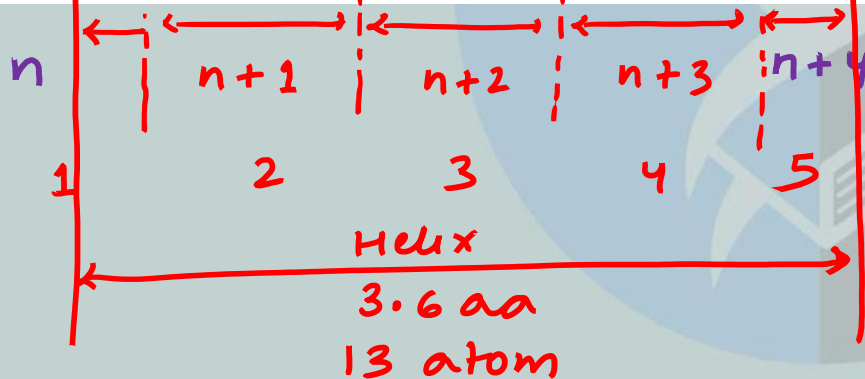
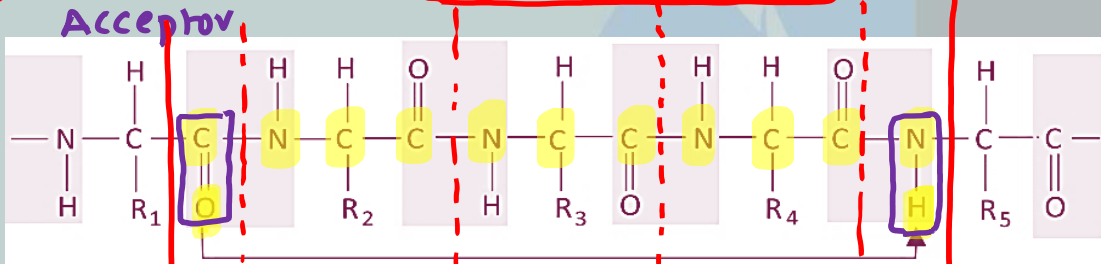


Hydrogen Bonds:

Main Chain C=O of $n \rightarrow$ N-H of $n+4$

Number of hydrogen bond: number of amino acids - 4

Side chain do not participate H-bonding



50 aa α -helix

$$\text{H-bond} : 50 - 4 = 46 \checkmark$$

α Helix
 3.6^{13} Helix

Ampipathic alpha helix

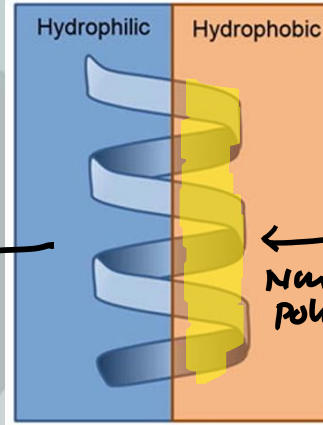
- val - asp - asp - val - phe - ser - gln - val - cys - thr
 - his - leu - asp - thr - leu - lys

eg Ion channel & Transporter

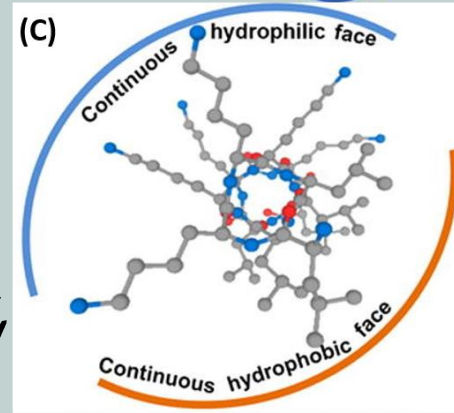
Polar

Non-Polar

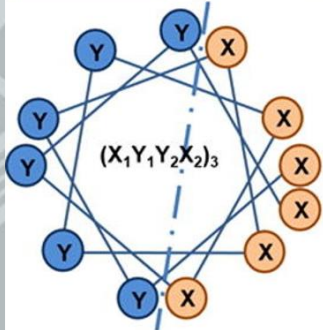
(A)



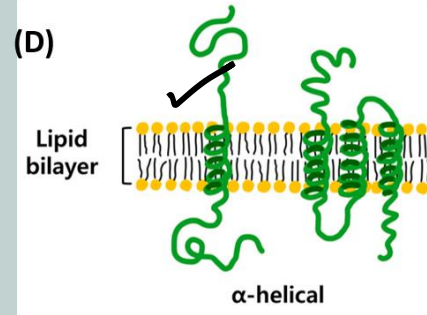
(C)



(B)



(D)



Hydrophobic alpha helix

- val - ala - ala - val - phe - met - gly - val - leu - ile
 - phe - leu - trp - ala - leu - val

eg. Transmembrane region of most of integral protein.

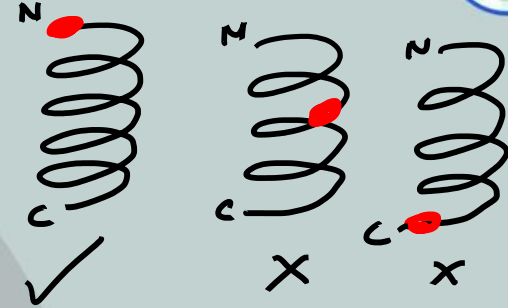


Disrupt α helix

Proline

Hydroxy-proline

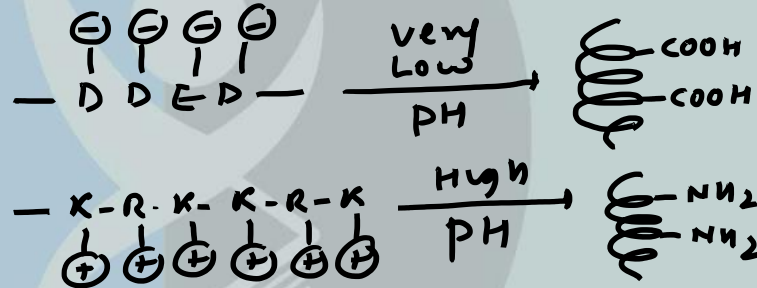
← cannot be seen in middle or c-terminus of α -helix



Destabilize α helix

↓ Asp, Glu

- Continuous acidic amino acids — Asp, Glu
- Continuous basic amino acids — Arg, Lys
- Continuous glycine
- Continuous large branched aa eg Isoleucine



example : α -Keratin, transmembrane region
myoglobin, Hemoglobin



What is the length and number of hydrogen bonds in a polypeptide with 20 amino acid residues in a single contiguous α -helix spanning hydrophobic core of plasma membrane?

- ✓ (1) 3 nm and 16
- (2) 2 nm and 16
- (3) 10.8 nm and 32
- (4) 30 nm and 32

$$\begin{aligned}\text{Length} &= 20 \times 0.15 \text{ nm} \\ &= 3 \text{ nm}\end{aligned}$$

$$\begin{aligned}\text{H-bond} &= \text{aa} - 4 \\ &= 20 - 4 \\ &= 16\end{aligned}$$

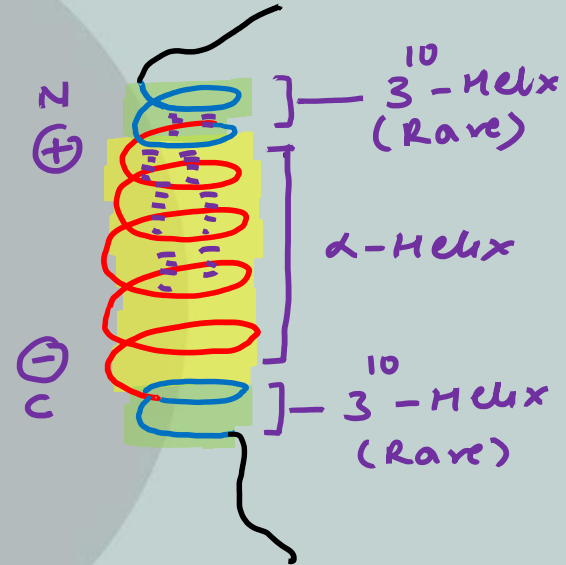


3_{10} -helix

- Observed as extensions of α -helices found at either their N- or C- termini.
- ϕ, ψ dihedral angles near $-50^\circ, -25^\circ$
- Right-handed helical structure
- $n \rightarrow n+3 \leftarrow \text{H-bonding}$
- Distance between two amino acids 2.0 \AA

(0.2 nm)

- 3 : Number of aa in 1 Helix
- 10 : Number of atom in 1 Helix



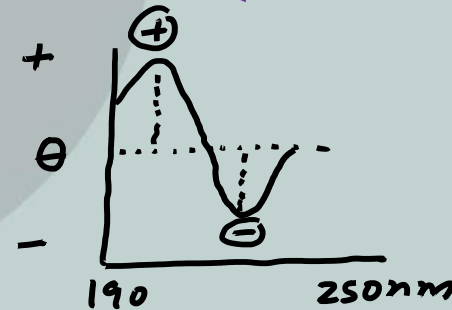


Beta Sheets:

- The β sheet structures are quite common in nature
- Generally seen in interior of globular protein
- Distance between two amino acids $3.5 \text{ \AA} = 0.35 \text{ nm}$

- Inter/intra chain hydrogen bonding
- ϕ, ψ are about $-140^\circ, +135^\circ$ in Antiparallel sheets
- ϕ, ψ are about $-120^\circ, +115^\circ$ in Parallel sheets

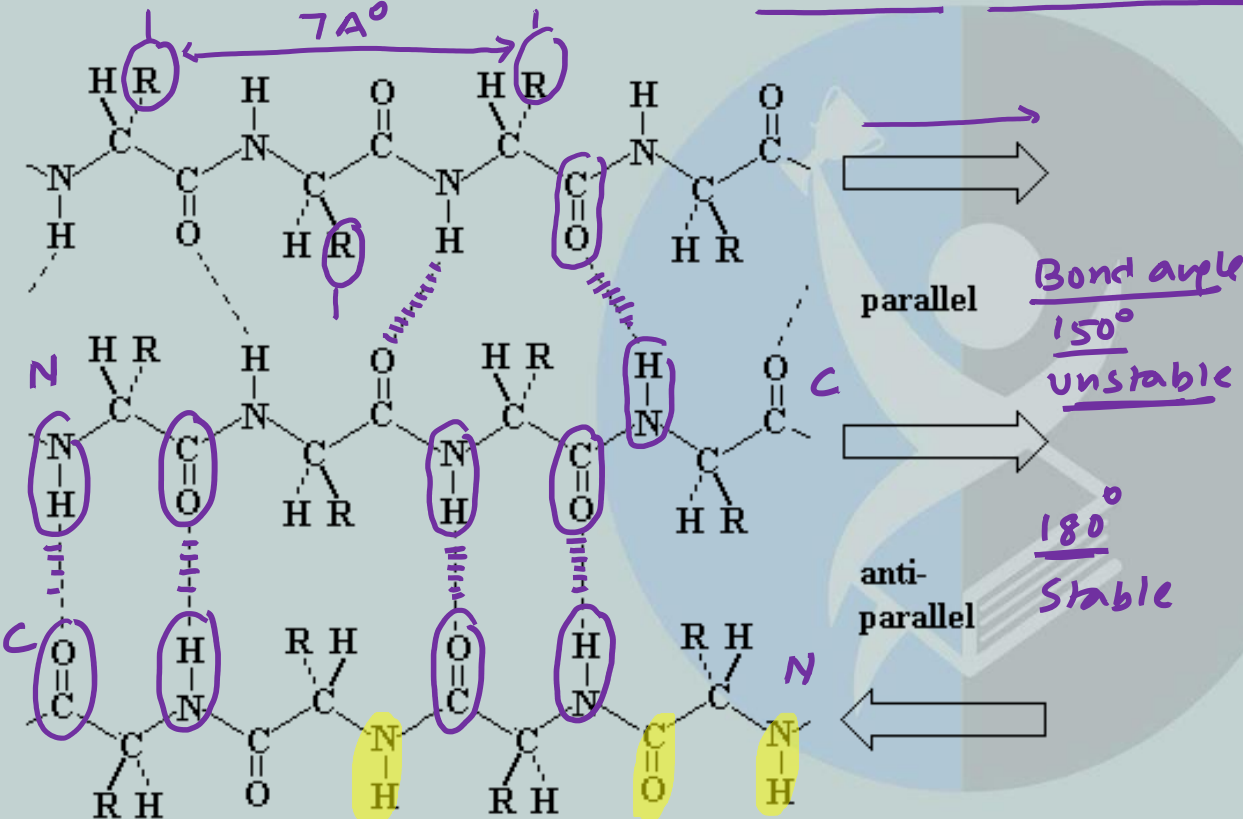
Far UV
CD
Spectroscopy



Distance betⁿ

	2 aa
π -Helix	1.25 \AA
Distorted α -Helix	1.45 \AA
α -Helix	1.5 \AA
3^{10} -Helix	2.0 \AA
β -sheet	3.5 \AA
Random	3.8 \AA

Hydrogen bonding occurs between neighboring polypeptide chains



3^{10} -helix

$n \rightarrow n+3$

α -helix

$n \rightarrow n+4$

π -helix

$n \rightarrow n+5$

Rare, unstable

Seen in middle of very long α -helix

- 50% Amide Hydrogen are involved in H-bonding
- 50% H-D exchange in NMR spectroscopy

Rare Amino Acids in Beta Sheets

Proline

Acidic \ominus

Basic \oplus

} Repulsion

Most Common

✓ Isoleucine

✓ Valine

✓ Alanine

• Small R-chain aa

Examples of β -sheets

eg 1. Silk fibroin (Antiparallel β -sheets)

2. Immunoglobulin fold

3. Porin membrane protein

β -Barrel

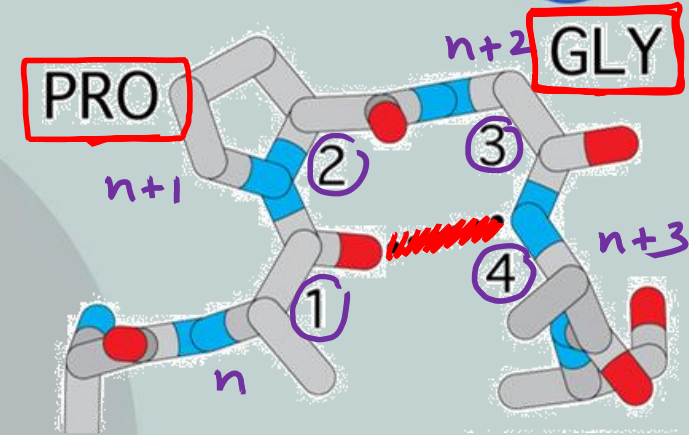
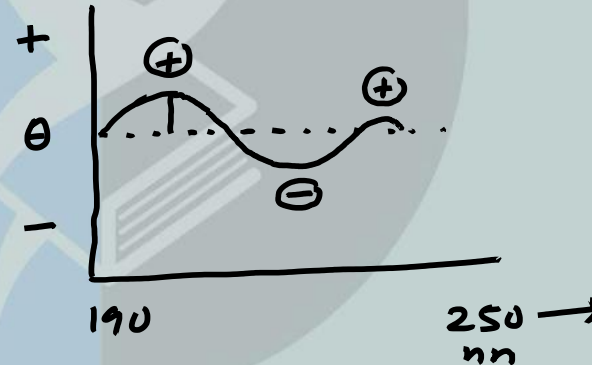
- outer membrane
- mitochondria
- chloroplast
- Gram -ve bacteria





β Turn or Reverse turn or Hairpin Bend:

- ✓ Formed by 4 amino acids
 - The CO group of residue 'n' is H-bonded to the NH group of residue n+3.
 - n+1 and n+2 do not participate in H-bonding
 - Different types on basis of ϕ and ψ values of n+1 and n+2 amino acids




 $n+1$
 $n+2$

	Residue 2		Residue 3	
	ϕ	ψ	ϕ	ψ
Type-I	-60	-30	-90	0
Type-I'	+60	+30	+90	0
Type-II	-60	+120	-90	0
Type-II'	+60	-120	+90	0

- Type of Reverse turn depends on ϕ & ψ values of residue 2 & residue 3

ϕ Proline

D-proline $\phi = +60$
L-proline $\phi = -60$

ψ is variable

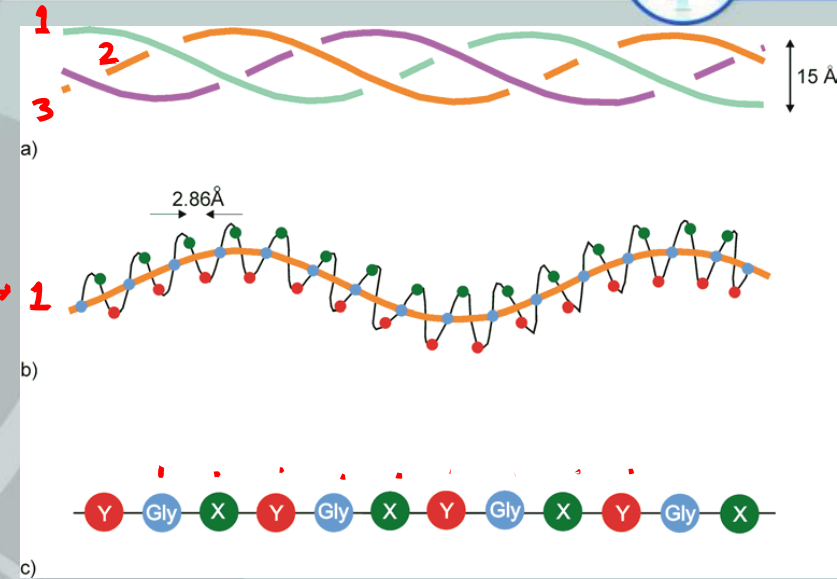
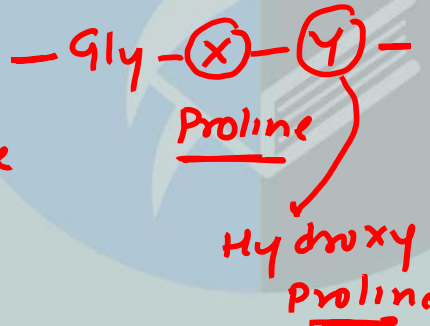


Collagen helix : α -polypeptide chains

- ✓ Gly-X-Y
- ✓ Left-handed helix
- ✓ Peptide bond: trans
- ✓ 3.3 residues per turn
- ✓ 2.86 (2.9) Å. ← Distance betⁿ 2 aa
- ϕ and $\psi = \underline{-51^\circ}, \underline{+150^\circ}$

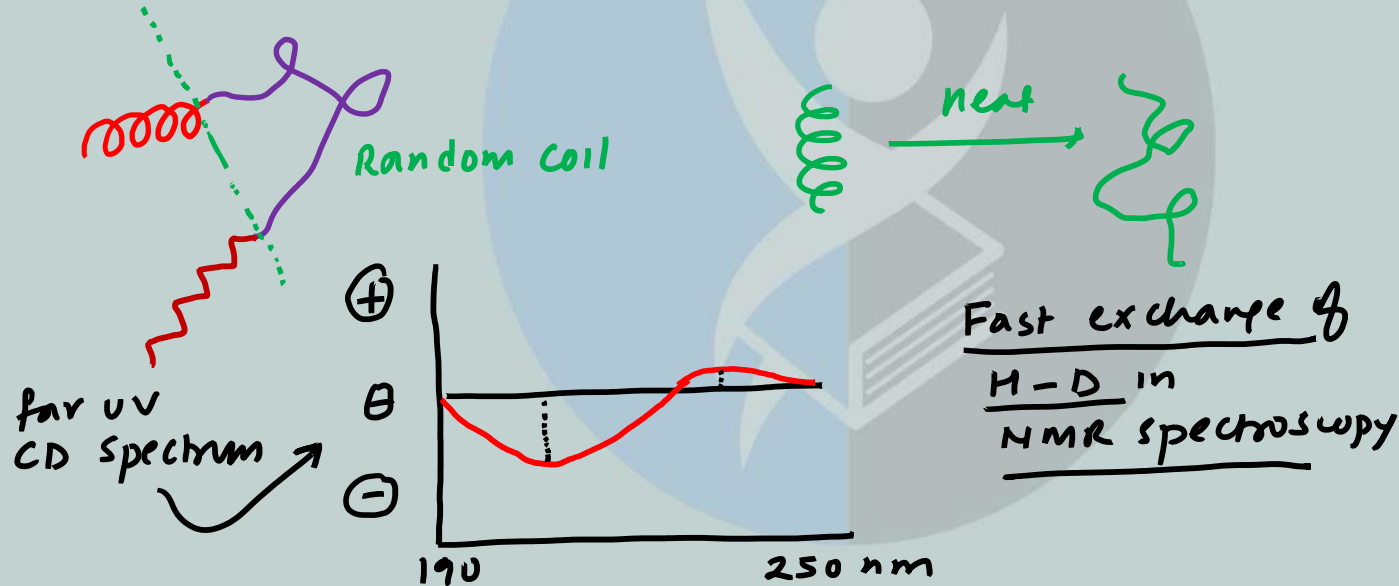
- Intra-chain H-bonding absent.

- Resembles poly-proline type II helix.



Random Coil

When a polypeptide contains adjacent bulky residues such as isoleucine or charged residues such as glutamic acid and aspartic acid, repulsion between these groups causes the polypeptide to assume a random coil configuration



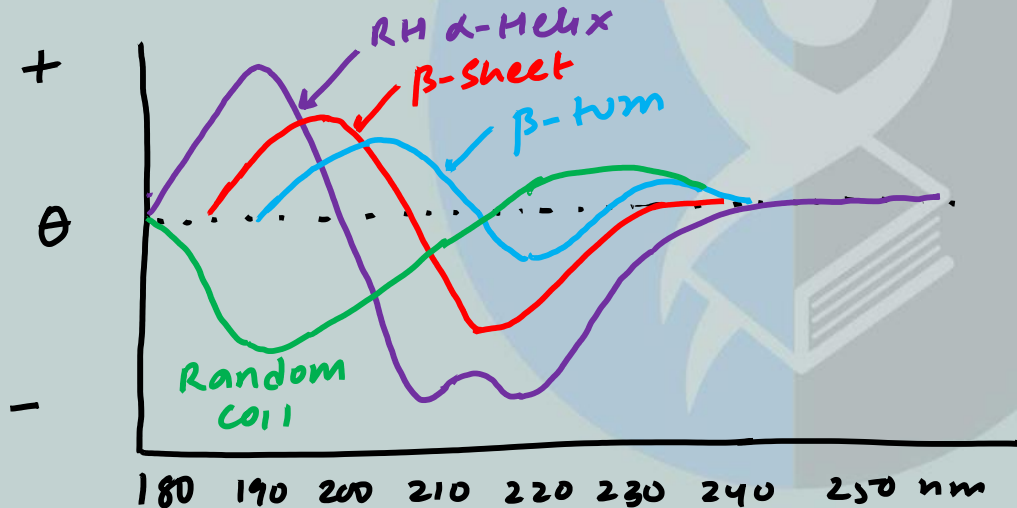
Techniques to study secondary structure of proteins

- ✓ 1. Far UV- Circular Dichroism (CD) Spectroscopy
- ✓ 2. H/D Proton Exchange NMR Spectroscopy
- ✓ 3. X-Ray crystallography
- ✓ 4. Fourier Transform Infra Red (FTIR) Spectroscopy

Fast Exchange : Random coil

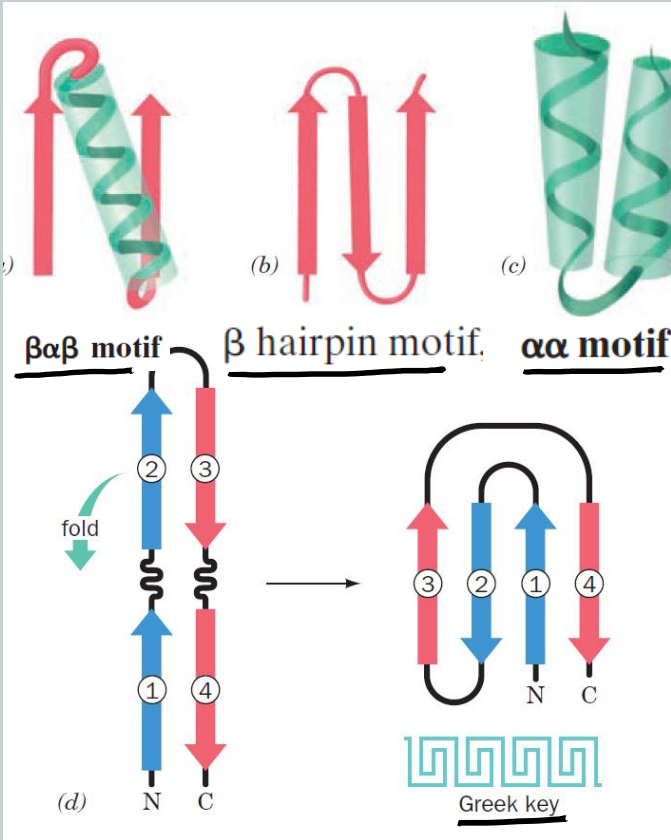
50% Exchange : β -sheet

Slow Exchange : α -helix





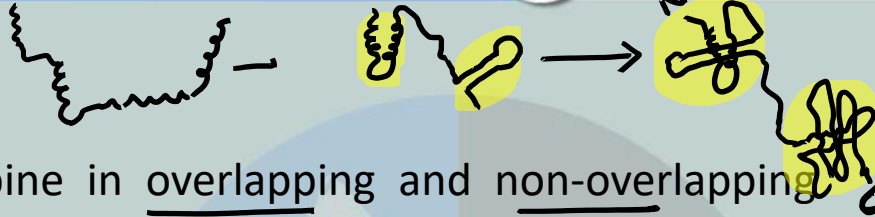
Super secondary Structures (Motif): building blocks for 3rd structure



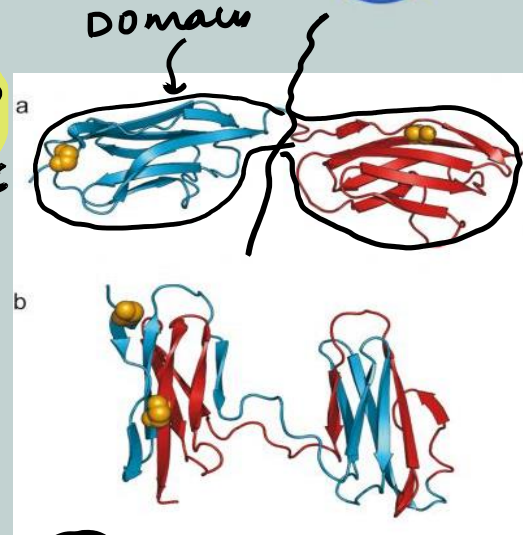
- ✓ Combinations of basic secondary structures linked together in a specific geometric arrangement.
- ✓ Fundamental building blocks that contribute to the overall 3D structure.
- ✓ They are more complex than simple secondary structures but smaller than tertiary structures.
- ✓ Have specific biological functions
 eg DNA binding protein
 - Helix turn Helix motif (Prokaryotes)
 - Leucine zipper motif
 - Zinc finger motif



Domain and fold

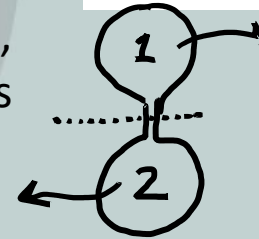


Groups of **motifs** combine in overlapping and non-overlapping ways to form the domain.



Domain is a conserved part of a given protein sequence and (tertiary) structure that can evolve, function, and exist independently of the rest of the protein chain.

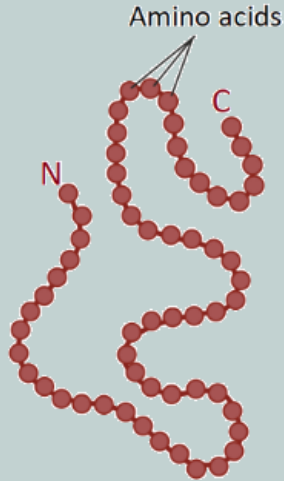
While a domain is a functional and structural subunit of a protein, a fold describes the arrangement of the secondary structures within the domain.



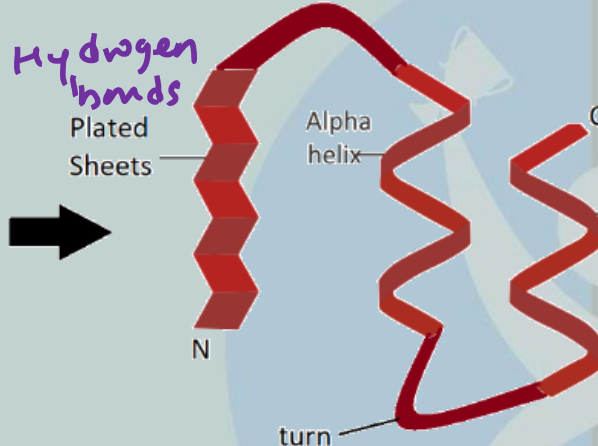
Common folds include the alpha/beta-barrel, beta-sandwich, and Rossman fold.



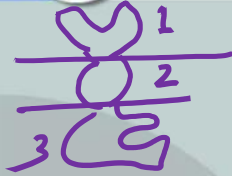
TERTIARY STRUCTURE OF PROTEINS:



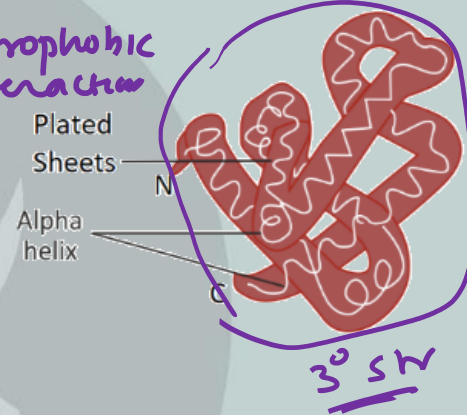
Primary Structure



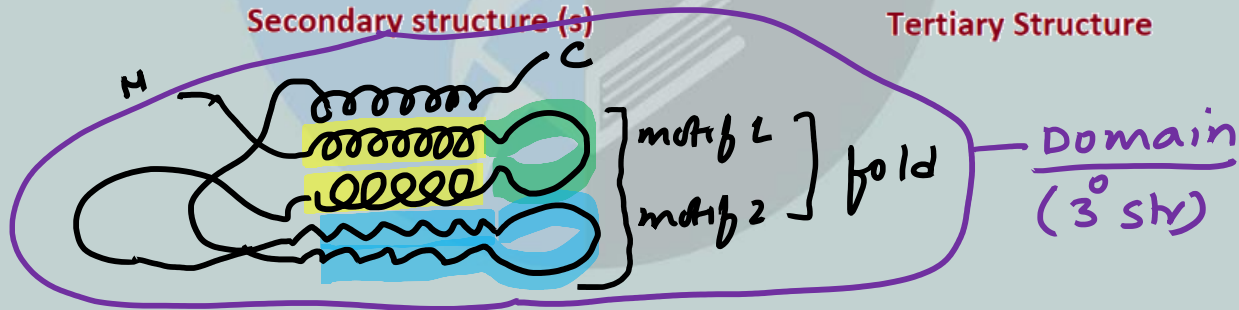
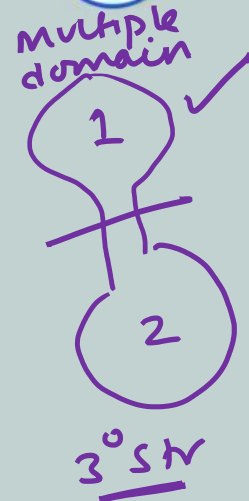
Secondary structure (s)



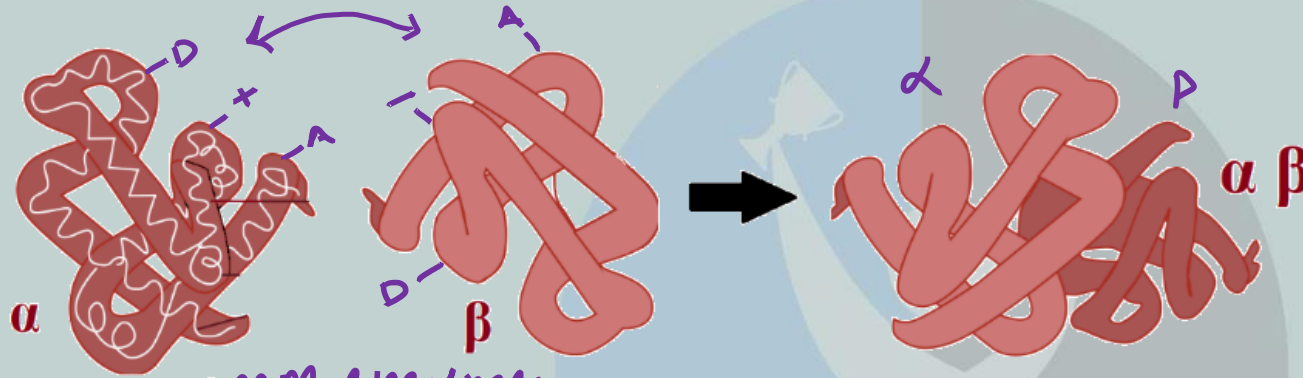
Hydrophobic Interaction



Tertiary Structure



QUATERNARY STRUCTURE OF PROTEINS:



3^o • MM enzyme
Tertiary Structure(s)

- Single subunit
- Albumin
- RNase A
- myoglobin
- cyt C
- insulin

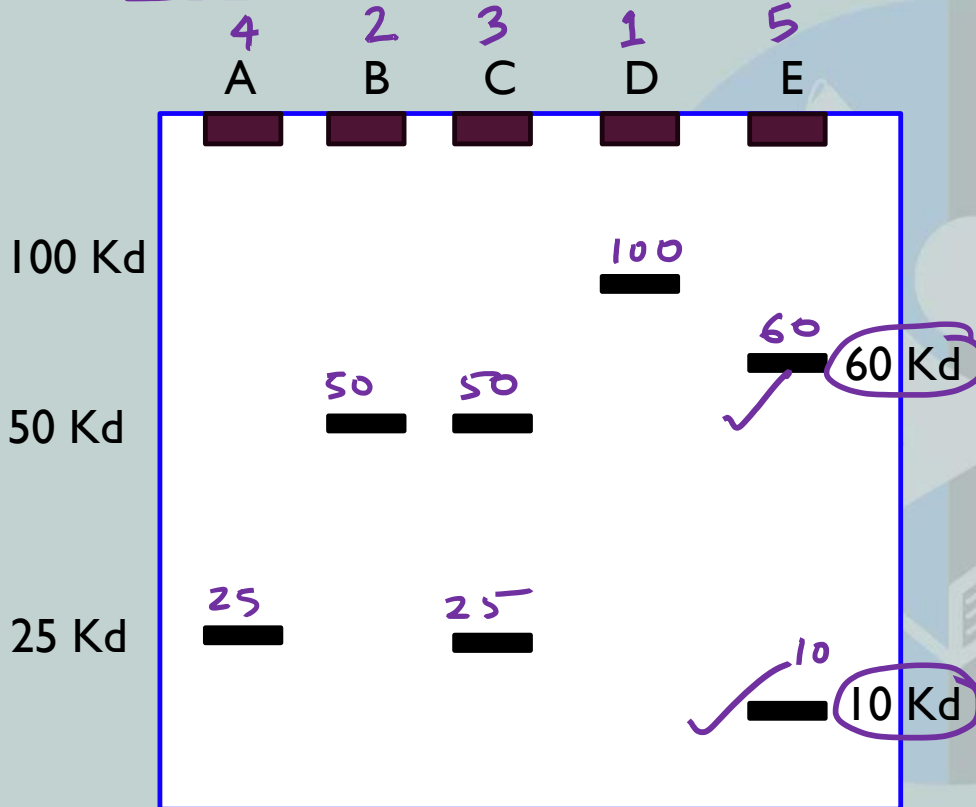
Quaternary Structure

- Multiple subunit
- RUBISCO
- Immunglobulin
- Hemoglobin
- RNA polymerase
- Allosteric enzyme



SDS Analysis under reducing condition for Assessment of Oligomeric Status

+ β -ME or DTT



- Non-covalent interactions are disrupted by SDS
- S-S bond is broken by reducing condition

(A)	(B)	(C)	(D)	(E)
25	50	50	100	60
25	50	25		10
25		25		10
25				10
100	100	100	100	100



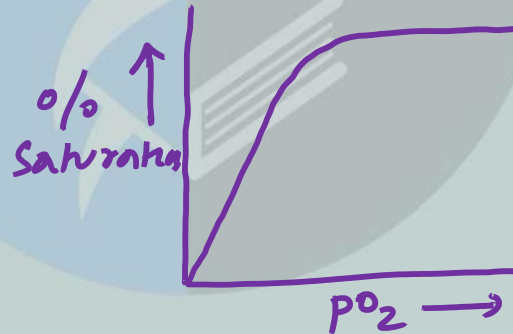
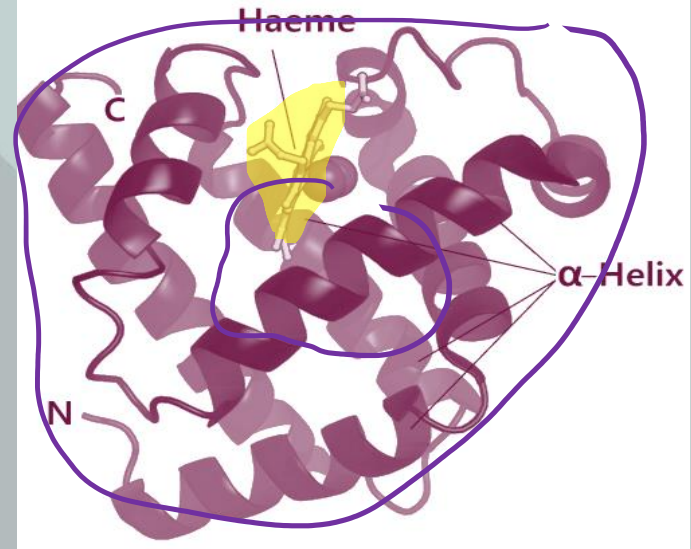
Globular Proteins:

I. Myoglobin

- ✓ 153 amino acids (16.7 kDa) heme protein.
- Contains alpha helix and loops
- High affinity for oxygen and serves primarily as an oxygen storage molecule
- Abundant in the muscles of diving mammals such as whales and seals.
- Tertiary structure
- Binds to single O_2 molecule

Core: Non-polar
Periphery: Polar aa

Hisidine — [Porphyrin-IX]
 Fe^{2+}
Heme



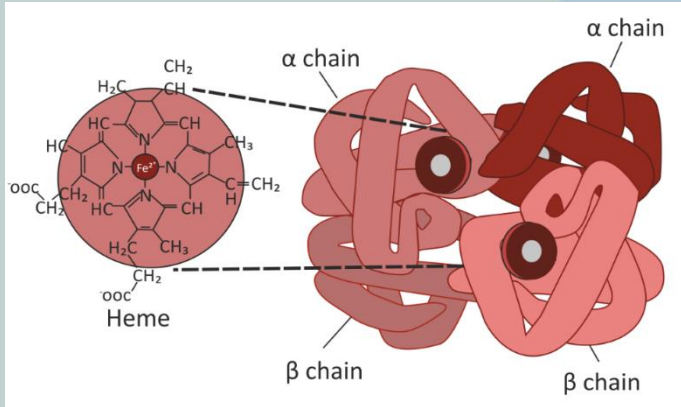


Haemoglobin

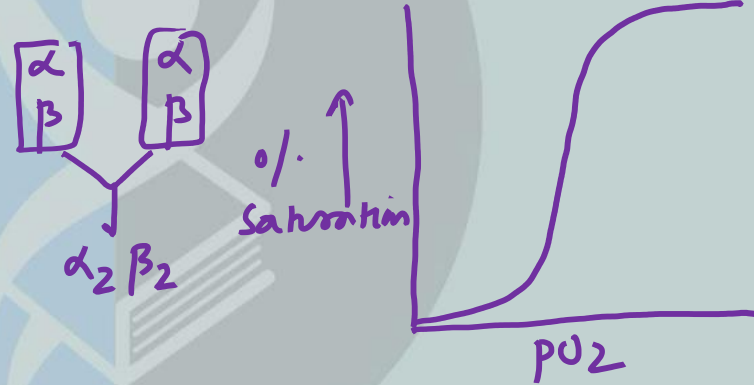
- Present in **RBC**
- Hemoglobin (64.5 kDa) contains **four polypeptide chains**
- Four heme** prosthetic groups having the **iron atoms** in the **ferrous (Fe^{2+}) state**.
- Exhibits **cooperative binding** of oxygen
- Transport of oxygen from the **lungs** to the **body's tissues**.

$2\alpha, 2\beta$: Adults (99%)
 $2\alpha, 2\delta$: Adults (1%)

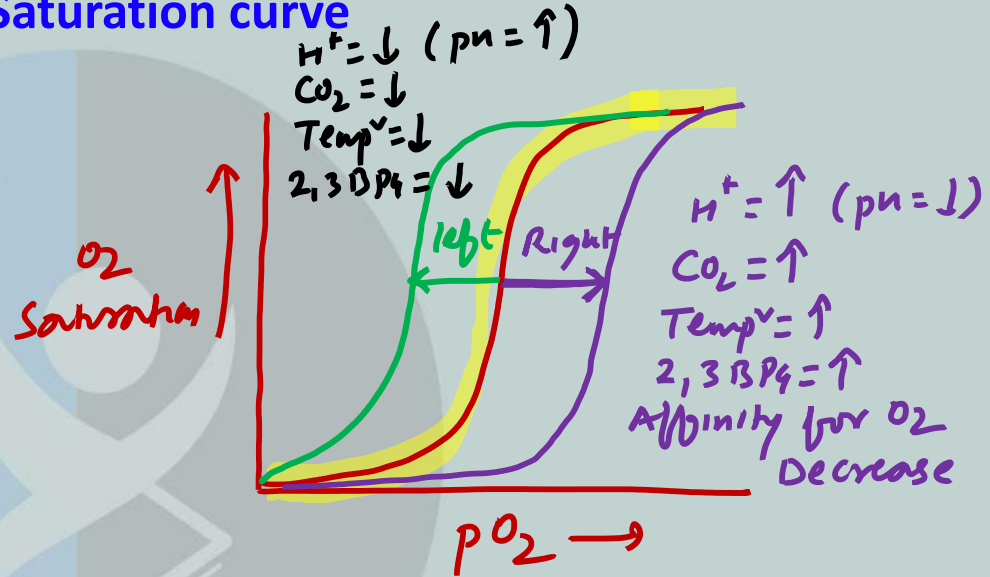
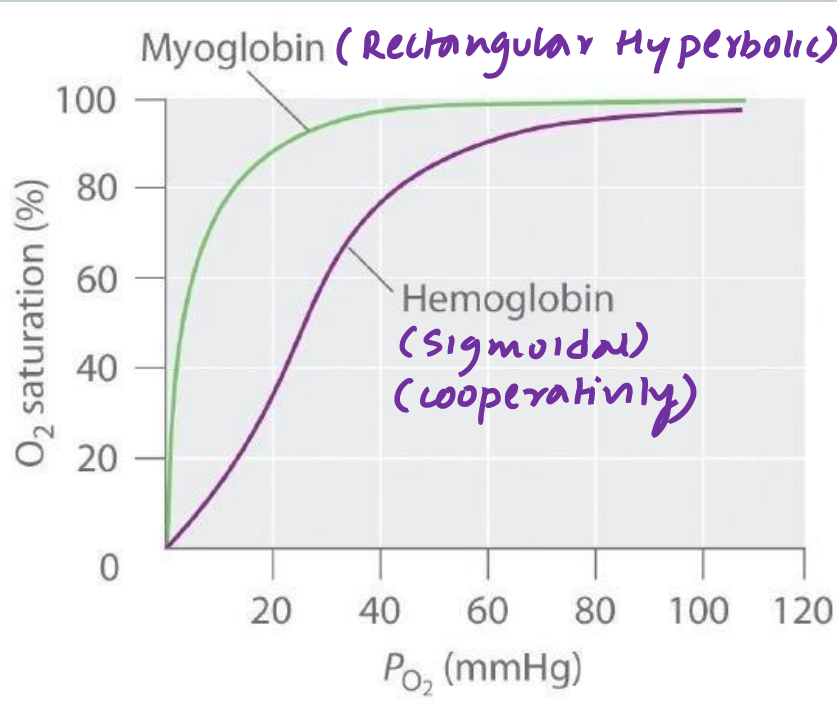
$2\alpha, 2\varepsilon$: Embryo
 $2\alpha, 2\gamma$: Fetus



Non-covalent
Interaction



Myoglobin and Haemoglobin Oxygen Saturation curve





Fibrous Protein : Abundant in hydrophobic amino acids

I. Keratin

Fibrous

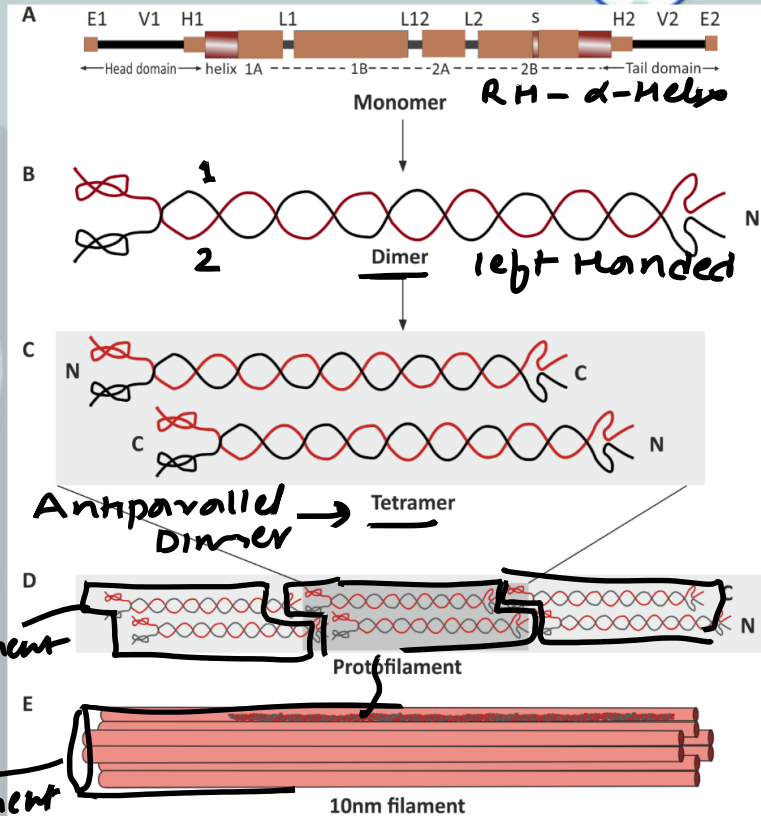
✓ Type of Intermediate filaments: provides mechanical support and protection against abrasion for epithelial cells.

✓ Have high content of cysteine, which forms disulfide bonds that contribute to the strength

✓ Alpha Keratin: Skin, Hair, Nail, Horn of Mammals
Beta Keratin: feathers, claws, beaks of birds & reptiles

Keratin Gene mutation

- Epidermolysis bullosa simplex (EBS)
- Epidermolytic hyperkeratosis (EHK)



Proto filament

Keratin filament

2. Collagen: Most abundant protein in the animal → matrix of bones, muscles
Basal lamina

Primary Structure : Gly -X -Y

	G	X	Y	G	X	Y	G	X	Y
958	Gly	Pro	Arg	Gly	Pro	Hyp	Gly	Ser	Ala
967	Gly	Ser	Hyp	Gly	Lys	Asp	Gly	Leu	Asn
976	Gly	Leu	Hyp	Gly	Pro	Ile	Gly	Hyp*	Hyp
985	Gly	Pro	Arg	Gly	Arg	Thr	Gly	Asp	Ala
994	Gly	Pro	Ala	Gly	Pro	Hyp	Gly	Pro	Hyp
1003	Gly	Pro	Hyp	Gly	Pro	Hyp	Gly	Pro	Pro

Post translational modification

Y: Proline → Hydroxy proline

Prolyl hydroxylase requires

✓ O_2 , ascorbic acid (vitamin C) and Fe^{2+} .
deficiency

Scurvy

Lysine → hydroxy lysine

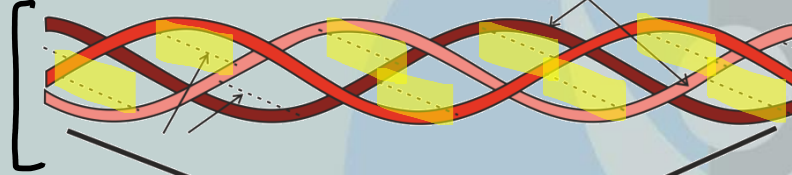
Primary
Structure

Gly-X-Y-Gly-X-Y-Gly-X-Y-Gly-X-Y-

Secondary
Structure



Polypeptide chains



Tropocollagen
~300 nm

Fibrils
~1 μ M



Collagen Fibers
~10 μ M



← collagen Helix
Left handed

$$\phi = -50 \quad \psi = +150$$

Super Helix
Triple Helix } 3-2- polypeptide chain

→ Right handed
→ inter chain H- bonding

• Covalent bonds

• merodesmosine bond or
lysine nor leucine bond



✓ Type of collagen depend on type of α polypeptide used for triple helix

At least 28 types of collagen

Type	Polypeptide composition	Distribution
✓ <u>I</u>	<u>$\alpha 1(I)_2 \alpha 2(I)$</u>	<u>Skin</u> , <u>bone</u> , tendon, blood vessels, cornea
II	<u>$\alpha 1(II)_3$</u>	<u>Cartilage</u> , intervertebral disk
III	<u>$\alpha 1(III)_3$</u>	<u>Fetal skin</u> , <u>blood vessels</u>
<u>IV</u>	<u>$\alpha 1(IV)_2 \alpha 2(IV)$</u>	<u>Basement membrane</u>
V	<u>$\alpha 1(V)_2 \alpha 2(V)$</u>	Placenta, skin



Collagen defects ← *Genetic disorder*

- Osteogenesis imperfecta (brittle bone disease) – deformation of bones
- Ehlers-Danlos syndrome – affects skin, joints, and blood vessel walls
- Scleroderma – hardening of the skin.



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